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This is a request for filing a **PROVISIONAL APPLICATION FOR PATENT** under 37 CFR 1.53 (b)(2).

Docket Number	25981	Type a plus sign (+) inside this box ->	+

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TITLE OF THE INVENTION (280 characters max)

PORTABLE PARTICLE-SOLUTION DELIVERY SYSTEM

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification	*Number of Pages*	10	☑ Applicant is entitled to Small Entity Status	
☑ Drawing(s)	*Number of Sheets*	3	☑ Other (specify) *ASSIGNMENT*	

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No

☐ Yes, the name of the US Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____ April 9, 2003 Date

TYPED or PRINTED NAME SOL SHEINBEIN 25,457 REGISTRATION NO. *(if appropriate)*

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PORTABLE PARTICLE-SOLUTION DELIVERY SYSTEM

Inventors: Rony Hemo, Itay Maoz, Yarden Dloomi andGal Yarden

Field of the invention:

The present invention relates to the fields of mechanical engineering and plant protection.

Background of the Invention:

There are currently over 70 different defined genera of plant viruses, containing more than 600 known species. Almost all plants both mono- or dicotyledon — are hosts to at least one virus. Plant viruses have a whole array of different particle morphologies, host ranges, vectors (for example, insects, nematodes, fungi, pollen, seeds or humans), genome organizations and gene expression strategies. They cause symptoms which at their least severe are unnoticeable, but range upwards through ring spots or mosaic leaf patterns, to widespread necrosis. Plant viral diseases cause severe economic burden to agriculture worldwide. Current practices for partially controlling viral epidemics include:

- Use of extremely toxic pesticides to control the vectors.
- Spraying of various oil formulations to decrease the ability of the vectors to interact with the plant.
- Physical barriers such as net houses that deny access of the pests.
- Use of transgenic varieties that are resistant to the virus.

However, the above methods are restricted in their capacity to confine viral epidemics in the following ways:

a) Pesticides cause great environmental damage, human morbidity and public anxiety. Indeed, the use of these highly toxic formulations is under global pressure for being phased out, especially in the developed world.

b) Oil spraying is labor-intensive and not totally effective.

c) Net-houses are useful, yet extremely expensive implying that outdoor field growing of certain crops is not practiced.

d) Genetically Modified (GM) plants are not globally accepted. In addition, the high mutation rates of the viruses often leads to collapse of resistance within a few years.

Although it has been known for many years, it is not widely appreciated that plants can be protected from a severe, deliberating virus by prior infection with a mild strain of a closely related virus. This is known as cross-protection. The principle of cross protection was discovered by McKinney (1929), when he observed that a tobacco plant systemically infected by a green strain of Tobacco mosaic virus (TMV, Tobamovirus) was protected from infection by another strain of this virus inducing a yellow mosaic. This phenomenon was subsequently observed for many other plant viruses and considered to be of interest for protecting plants in the field. The principle was that an 'infection of a plant with a strain of virus causing only mild disease symptoms may protect it from infection with severe strains' (Matthews, 1991). Gonsalves and Garnsey (1989) introduced a more applied definition of cross protection as 'the use of a mild virus isolate to protect plants against economic damage caused by infection with a severe challenge strain of the same virus'. In this case cross protection is mainly evaluated by the farmer's economical benefits rather than defined by a specific mode of action or by a type of interaction between virus strains. Cross-protection has received a lot of attention in the literature and several hypotheses were conjured in order to explain how a particular strain of virus confers relative protection against a similar more virulent strain. In essence it has been observed that only viral strains that are closely related to the virulent virus confer resistance and that more distantly related strains are incapable of eliciting this phenomenon (Desbiez and Lecoq 1997). Initially, the coat protein was implicated as playing the pivotal role in cross-protection. Today, there is a growing body of evidence to support the fact that at least for several plant viruses, at least part of the mechanism underlying cross-protection is a post-transcriptional and RNA-mediated process that targets the secondary-challenged virus in a nucleotide-sequence-specific manner (Waterhouse et al. 2001, Voinnet 2001).

In one plant virus family, the potyviruses, there is at least one known determinant for symptom severity. For example, the single mutation FRNK -> FINK in the helper component viral protein (HC) confers mildness of the symptom without affecting the replication (Gal-On and Raccah 2000). A naturally mild isolate of Zucchini Yellow Mosaic Virus (ZYMV-WK) bearing this mutation was recognized several years ago by Dr. Herve Lecoq (INRA, France). Economically relevant cross-protection has been achieved with this naturally occurring mild ZYMV (Yarden et al. 2000). Moreover, this mutation can be introduced to infectious potyvirus recombinant clones by directed mutagenesis in order to engineer attenuated clones, and these are also useful for cross protection.

Commercial application of cross-protection for potyviruses requires the safe introduction of these mild isolates. Thus, it is imperative that once introduced, these viruses will be confined only to the protected plant and not be inadvertently transferred by the viruses vectors, namely the leaf aphids. Determinants affecting aphid transmission are also known. Indeed, one mutation in the coat protein (CP) (namely DAG ->DTG Atreya et al. 1990, Gal-On et al., 1992), and two in the HC (KLSC->ELSC Atreya et al., 1992 or PTK ->PAK, Huet et al., 1994) totally abolish the transmission in this plant-virus family. Thus it is possible to naturally isolate or design a recombinant potyvirus mutant which contains any or all of the above mutations, and which will be absolutely non-aphid transmissible (NAT) and thus safe to introduce into the environment.

Previously, mild viruses introduced for cross-protection had to be inoculated by hand-rubbing mechanical friction. This confined the number of plants that could be infected and restricted the use of the method to instances where labor costs are negligible or the value of each plant is high, with no alternative method existing for protection. Moreover, the inoculation efficiency (i.e. the percentage of plants successfully infected) of the hand-friction method is usually not high enough for commercially relevant protection. Thus, the introduction of a multi-barrel plant inoculation gun (PCT/IL/00/00039), facilitated, for the first time, robust inoculation of up to 250,000 plantlets in a working day, compounded by very high efficiencies. However, this method is restricted to use in nurseries,

where trays bearing plantlets are transported on a conveyer belt and are subject to jet-propulsion from the machine as described therein. In addition this method can only be applied to young plantlets before transfer to the field, and cannot be applied to plants directly sown in the field or to for example, fruit trees.

Apparently, plants naturally defend themselves by exploiting the requirement of most plant viruses to replicate using a double-stranded replicative intermediate. A conserved biological response to double-stranded RNA, known variously as RNA interference (RNAi) or post-transcriptional gene silencing (PTGS), mediates plant resistance to exogenous pathogenic nucleic acids (mostly viruses) as well as endogenous parasitic sequences (mostly transposable elements), thus regulating the expression of protein-coding genes. Most plant viruses, such as the potyvirus family have ssRNA genomes and replicate in the cytoplasm using an RNA-directed RNA polymerase (RDRP) to produce both sense and antisense RNA. Therefore, replication of a ssRNA plant virus can produce sufficient stretches of dsRNA to be recognized as such within the plant. Genetic and biochemical studies have now confirmed that RNAi, PTGS and also co-suppression and cross-protection share mechanistic similarities and common biological pathways. Indeed, it has recently been confirmed that dsRNA-induced gene silencing exists in many, if not most, eukaryotic organisms including mammals. Most recently, direct biolytic delivery of dsRNA was shown to inhibit virus infection for several viruses from different genera (Tenllado and Di´Az-Rui´Z 2001).

There is thus a widely recognized need for, and it would be highly advantageous to have a portable device, which will enable to either cross-protect plants directly in the field or to deliver other particles such as dsRNA for direct protection from plant pathogens.

Summary of the Invention

In its various embodiments the present invention provides a portable device for rapid, reliable particle delivery for field-based plant inoculation comprising:

1) A liquid container containing the inoculating particle solution.

2) A compressed gas source.

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3) An inoculation discharge unit comprising a gas reservoir having a gas inlet connected to said compressed gas source and connected to a gas quick-exhaust valve.

4) A control unit for triggering the quick-exhaust valve.

5) A discharging unit for precisely directing the discharged inoculating solution onto a particular leaf or leaves of said plants.

Wherein the liquid container is connected via a pipe to the inoculation discharge unit and triggering the quick-exhaust valve causes condensed gas within the gas reservoir container to be released through the gas outlet, said discharged compressed gas flows into said discharging unit, said unit connected by a narrowed outlet to the flowing inoculating solution enabling directed discharge onto a leaf or leaves of said plants.

According to one particular embodiment the portable liquid container containing the inoculating solution is equipped with an internal mixer keeping the solution heterogeneous.

In a particular preferred embodiment, the heterogeneous solution consists of virus homogenate, buffer and carbordrum to facilitate virus entry.

In still another preferred embodiment the container is equipped with a pressure valve and an gas flow meter that regulate the pressure in the container according to the required flow rate of said heterogeneous solution upon entry to said control unit for triggering the quick-exhaust valve.

According to another embodiment the inoculation discharge unit comprises a handle consisting of said regulating solution flow rate.

In a further particular preferred embodiment the regulation solution flow rate regulates the solution flow to approximately 1 liter per hour.

In still another preferred embodiment the handle has a manual tap to stop solution flow when discharge is not practiced.

According to another preferred embodiment the inoculation discharge unit is built from a barrel comprising a reserve gas compartment.

In a particular preferred embodiment the barrel is made from bronze or stainless steel.

According to another preferred embodiment the quick-exhaust valve receives commands from an external valve.

In a particular preferred embodiment said external valve commands the quick-exhaust valve so as to cause a reduction in the gas pressure at the head of the quick-exhaust valve, which enables immediate evacuation of the gas reservoir towards the discharge unit.

In still a further particular preferred embodiment the discharge unit is composed of a nozzle that vacuum sucks solution from the heterogeneous mixture via the action of the enhanced gas flow resulting in said heterogeneous mixture being thrust onto the nozzle partition.

According to another preferred embodiment, the gas reservoir is refilled with gas after said immediate evacuation.

In a preferred embodiment said refilling of the gas reservoir by the said compressed gas source is facilitated by a non-return valve that prevents reverse gas flow upon said immediate evacuation.

According to another preferred embodiment the whole system is regulated by a control unit.

In a preferred embodiment said control unit regulates said discharge rhythm.

In still another preferred embodiment said control unit regulates said mixing of said heterogeneous solution.

In a further preferred embodiment said regulation of said discharge rhythm and said mixing of said heterogeneous solution is done with synchronization to the spread of plants in the field and the pace of flow in the nozzle.

Detailed description of the drawings:

Figure 1: Overview of the invention.

Figure 2: Describes the pressurized portable liquid container containing the inoculating particle solution.

Figure 3: Describes the discharge mechanism combining the compressed gas and heterogeneous solution for the actual particle discharge.

Figure 1: Is a general depiction of the invention describing the interaction between the different parts of the system.

The whole system is regulated by a central control unit (6). The portable liquid container (8), holds the inoculating particle solution, and dispenses the solution at a regular flow rate towards the inoculation discharge unit (11). The solution in the container is continuously stirred within the portable liquid container (8) to disable particles within the solution from sinking to the bottom. The rhythm of mixing is controlled by the control

unit (6) via a valve (7). Compressed gas flows through a pipe (9), into the portable liquid container (8) and into the inoculation discharge unit (11). The solution coming out of the portable liquid container flows to the inoculation discharge unit (11) through the solution pipe (10) on its way to the inoculation pipe (1), passing on the way the flow rate regulator (12) that controls the flow to required flow rate and from there to the cut-off switch (3) enabling to stop flow when work is not performed. After passing the cut-off switch (3) the liquid flows into the inoculation pipe (1). The gas flow begins from the pipe (9) and is governed by the valve (5), whose timing is regulated by the control unit (6) such that gas flows into the inoculation discharge unit (11) and from there to the inoculation pipe (1). Electrical input into the system can be from a D.C. or A.C. input (13), with a cut-off switch (2) mounted on the inoculation discharge unit (11), enabling switching the machine on and off.

Figure 2: Describes the pressurized portable liquid container containing the inoculating particle solution.

The inoculating particle solution is inside the pressure resistant container (31), wherein lies the mixing unit (30) that goes up and down by the action of a piston (23), thus forming a vortex within the container and prevents the sinking of the particles within the solution. The mixer is regulated by a valve (25) and an electrical helix (26), receiving its instructions from the control unit through an electrical cable (27). The pressurized container receives its gas flow through an auxiliary pipe (21), which passes through the pressure valve (22) with a pressure meter that regulates the pressure to the required level in the inoculation particle solution container (31). The pressure in the said container causes the solution to exit through the exit tubule (29) and from there to the pipe (28) that leads the solution into the fast discharge inoculation unit.

Figure 3: The body of the barrel (47) is a hollow pipe made of bronze or stainless steel, containing the inner mechanism and constituting the

gas reservoir during discharge. An gas pressure meter (45) is mounted on the barrel (47), giving a continuous indication to the pressure at any given time. Gas enters into the barrel through a pipe (46) with a dual intent: a) To refill the reservoir after discharge through a unidirectional valve (43), b) To stop gas being discharged through the fast-discharge unit (42), by creating a high pressure at the entrance of the quick-exhaust valve (49), and enabling gas discharge through the exit only (50). When the system is triggered the pipe (46) is exhausted enabling a fast pulse of gas to move from entrance (49) to the exit (50), and immediate emptying of gas from the reservoir (47). Gas being discharged from the exit (50) enters a venturian nozzle (41). This nozzle is built such that a small corridor to the solution pipe (44) enables the fast gas discharge to vacuum-suck inoculation solution and its subsequent direction onto the leaf.

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Claim:

1. Portable particle-solution delivery system essentially as described and exemplified herein.

Fig 1

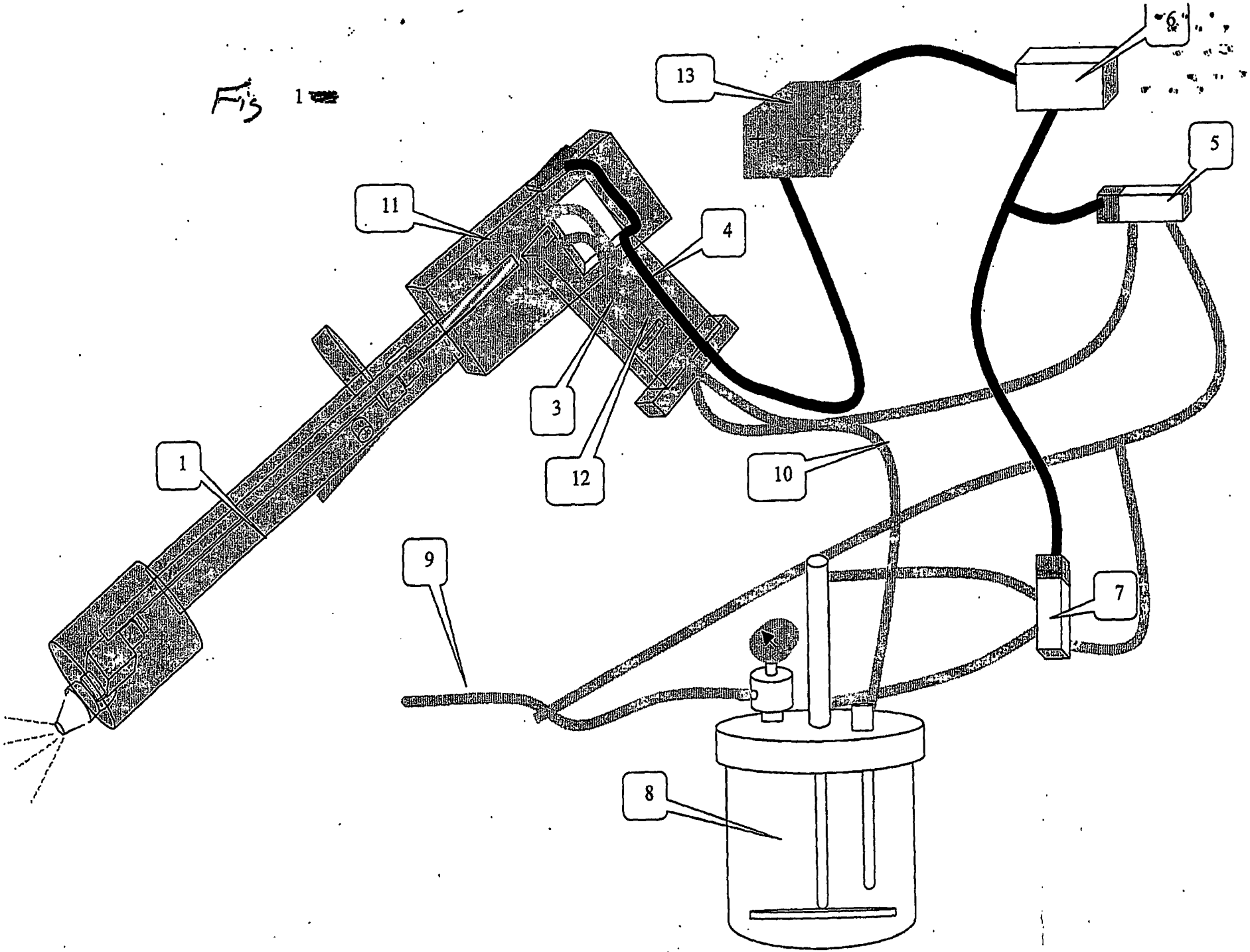

Fig. 2

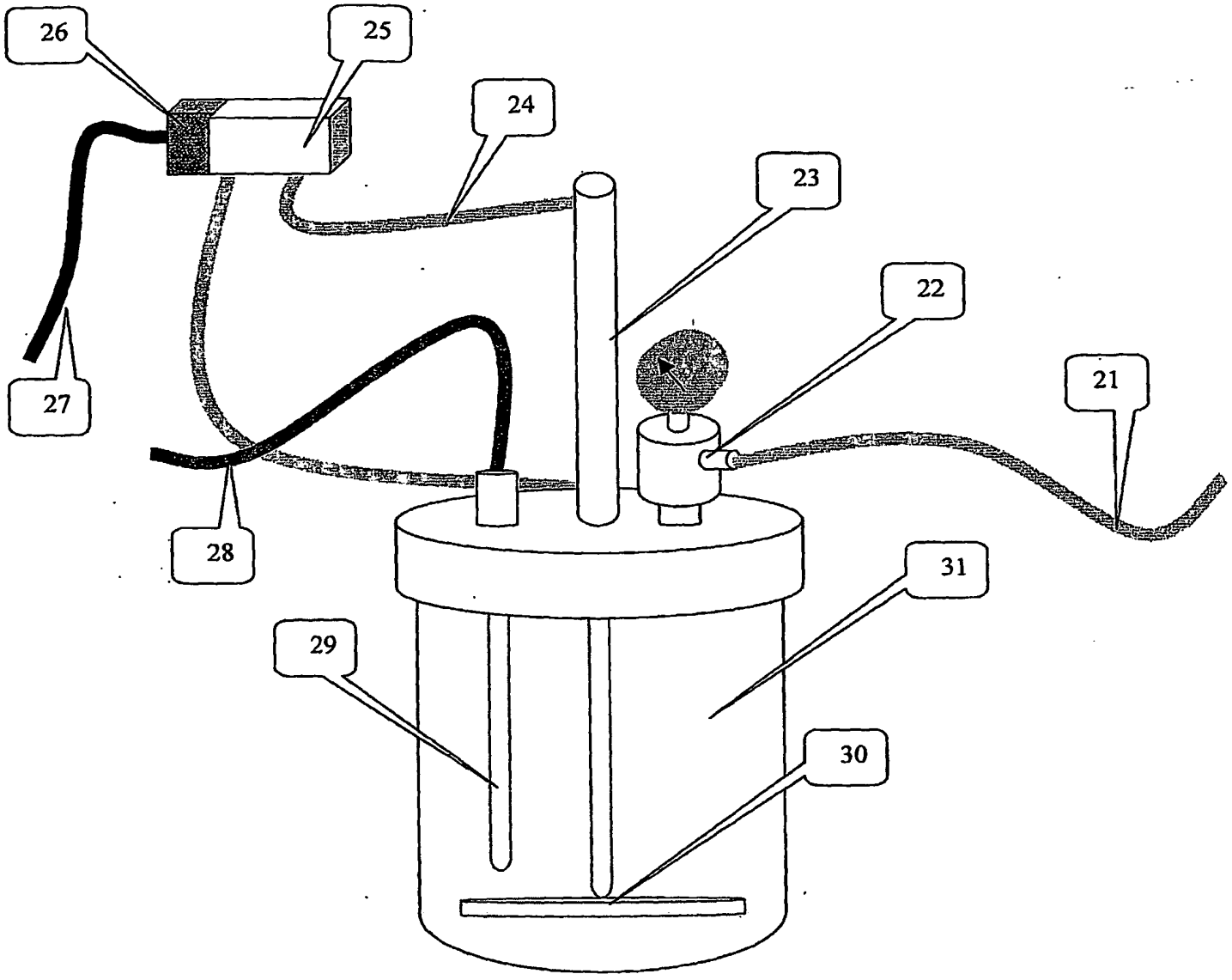

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Fig. 3